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Serum concentrations of fibrosis markers in children with inflammatory bowel disease

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Abstract: Background and study aims: The aim of the study was to assess the usefulness of serum concentrations of YKL-40/ CHI3L1 (a 40-kilodalton glycoprotein also referred to as chitinase 3 like-1 — CHI3L1) and PIIINP (N-terminal propeptide of type III procollagen), markers of fibrosis, in the monitoring of inflammatory processes and fibrosis in children with inflammatory bowel disease (IBD).
Patients and methods: In 60 patients (41 with Crohn's disease (CD), 19 with ulcerative colitis (UC)) concentrations of investigated parameters were measured at baseline (day 0), after 3 and after 6–8 weeks of pharmacological treatment.
Results: PIIINP concentrations were significantly higher in CD patients compared to UC (baseline results: median concentrations 1013.73 vs 78.30 ng/mL; P = 0.06 for the Kruskall-Wallis test; results at 6–8 weeks: 1076.48 vs 53.10 ng/mL, P = 0.01).
Fibrosis was clearly present in patients with CD and its severity increased (reflected by both YKL-40/ CHI3L1 and PIIINP concentrations) in 6–8 weeks of follow up, regardless of the treatment used during that time. In patients with UC the levels of YKL-40/CHI3L1 and PIIINP were lower at baseline and further decreased after 6–8 weeks (median concentrations were respectively: 39.5 ng/mL vs 24.7 ng/mL and 78.3 ng/mL vs 53.1 ng/mL).
Conclusion: Fibrosis was more severe in CD than in UC patients. The marker that more accurately reflected these differences was PIIINP.

Keywords: YKL-40/CHI3L1, PIIINP, Crohn's disease, ulcerative colitis.

Introduction

It is generally accepted that location and severity of inflammatory lesions are responsible for different clinical features and course of ulcerative colitis (UC) and Crohn's disease (CD). In patients with UC chronic inflammation of intestinal submucosa with deposition of collagen and development of fibrosis affects superficial layers of mucosa. In contrast, in patients with CD the inflammatory infiltrate may involve the whole thickness of intestinal wall with full-thickness collagen deposition and fibrosis leading to intestinal strictures.

YKL-40/CHI3L1 and PIIINP (N-terminal propeptide, of type III procollagen) may be useful for monitoring of clinical activity of inflammatory bowel disease (IBD) and prediction of fibrosis. YKL-40, also referred to as Chitinase 3-like 1 (CHI3L1) is a 40-kD glycoprotein known from early 1990s, when a radioimmune (RIA) assay measuring serum levels of YKL-40 was first used and described by Johansen, and its biological significance became better understood [1]. Although the role of YKL-40/CHI3L1 is still not fully established, its activity has been reported in many diseases, particularly those associated with inflammation and/or fibrosis. According to reports by Koutroubakis and Vind published in 2003 [2, 3] patients with IBD have higher serum levels of YKL-40/CHI3L1 than healthy subjects, and the levels correlate with clinical severity of the disease. Similar conclusions were drawn from preliminary studies of patients with CD. No data assessing the levels of these factors in children are available, except for one report by Wewer [4]. The role of PIIINP, both its metabolites and enzymes involved in its synthesis and degradation, in pathogenesis of IBD is not fully understood, although it is known that PIIINP activity is significantly higher in patients with UC. The relevance of PIIINP in pediatric IBD is also not known.

The aim of the study was to assess the usefulness of measurements of serum levels of YKL-40/CHI3L1 and PIIINP as biochemical markers of severity of intestinal fibrosis associated with inflammation in patients with IBD during first 8-weeks of treatment. Another aim of the study was to reveal the differences in the dynamics of fibrosis between UC and CD patients.

Patients and Methods

To this prospective controlled study we enrolled newly diagnosed patients with IBD, who fulfilled following inclusion criteria:

1. Age ≤18 years.
2. Children with IBD diagnosis confirmed by clinical features, endoscopic and histological examination.

3. Patients with a first episode of IBD with local and systemic manifestations (abdominal pain, diarrhea, blood in stools, fever, malaise).
4. Informed consent given by parents/guardians, as well as by the patient, if aged >16 years.

Control group consisted of 32 healthy children without gastrointestinal and fibrotic disorders. All participants didn't met exclusion criteria:

1. Decline to provide informed consent by parents/guardians, as well as by the patient, if aged >16 years.
2. Serious comorbidities (eg. pneumonia or other serious inflammatory disorders).

Baseline measurements of the levels of fibrosis factors were performed at the time of inclusion (i.e. diagnosis) in IBD patients, followed by the assessment at third and then sixth to eight week of treatment. In children from the control group the measurements were performed only once.

All IBD patients enrolled in the study underwent a standard diagnostic workup. In the majority of patients this included fecal occult blood test, microbiological examination of the stool (including pathogenic *Eschericha coli* [*EPEC*], *Salmonella spp.*, *Shigella spp.*, *Clostridium difficile*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica*), parasitological examination of the stool (including Giardia intestinalis antigen) and mycological examination of the stool followed by gastrointestinal endoscopy (duodenoesophagoscopy, ileocolonoscopy) and histological examination of biopsy specimens. Assessment of severity of the disease included clinical manifestations and complete blood count (CBC), erythrocyte sedimentation rate (ESR) and serum levels of C-reactive protein (CRP), albumin and total protein.

The baseline data on signs, symptoms and treatment were collected using a dedicated questionnaire for children with IBD.

The study was approved by Medical Ethics Committee of Jagiellonian University in Cracow decision: No. KBET/6/B/2009 of 29 January 2009.

Biochemical studies

Serum concentrations of YKL-40/CHI3L1 and PIIINP were measured in Clinical Biochemistry Department, Institute of Paediatrics, Jagiellonian University Medical College, using ELISA assay (METRA YKL-40 ELISA Kit, Quidel; and PIII-NP ELISA Kit, Wuhan USC Science ltd).

Statistics

The levels of YKL-40/CHI3L1 and PIIINP in respective time points were compared using t-Student test for dependent variables. For comparisons with the control group the exact Fischer test, t-Student test for independent variables, or Kruskall-Wallis test were used.

Statistical calculations were performed using IBM SPSS Statistics 21 software. The level of statistical significance was assumed at the risk of the Type I error below 5% ($\alpha = 0.05$).

Sample size was calculated on the basis of literature data stating that mean level of YKL-40/CHI3L1 in the population of patients with IBD is 90 ng/mL+/–80(SD). The change of 40 ng/mL during treatment was assumed clinically significant. To document this difference with 5% probability of Type I error and 80% statistical power, the study should include at least 60 children, which was achieved.

Results

92 children (48 girls and 44 boys) aged from 2 to 18 years (mean age 12.86 years, SD ± 4.09, median age 14 years) were enrolled in the study. Clinical characteristics of the study group is given in Table 1.

Table 1. Characteristics of IBD population. Data given in table were completed at the time of informed consent.

Patient ID	Gender	Age	Diagnosis	PCDAI/ PUCAI	Location of inflammation	Medications
CD-1	M	15.5	CD	57.5	L4	AZA, CS
CD-2	M	15	CD	25	L1	5-ASA
CD-3	F	2	CD	30	L2,L4	5-ASA, AZA, CS
CD-4	F	13.5	CD	32.5	L2,L4, p	5-ASA, AZA, CS
CD-5	F	17	CD	15	L3	5-ASA, CS
CD-6	M	12	CD	25	L1	5-ASA, AZA, CS
CD-7	F	10	CD	25	L3	5-ASA, AZA, CS
CD-8	F	8	CD	35	L3, L4	5-ASA, CS
CD-9	M	12.5	CD	10	L3, p	5-ASA, AZA, CS
CD-10	M	14	CD	10	L1	5-ASA
CD-11	M	16	CD	22.5	L3, p	5-ASA, AZA
CD-12	M	6	CD	30	L2, L4	5-ASA, AZA, CS
CD-13	M	14	CD	5	L3, p	AZA

Table 1. Cont.

Patient ID	Gender	Age	Diagnosis	PCDAI/ PUCAI	Location of inflammation	Medications
CD-14	M	8	CD	20	L1, L4	5-ASA
CD-15	F	16	CD	5	L3	5-ASA, AZA
CD-16	F	18	CD	40	L3, L4, p	5-ASA, IFX, CS
CD-17	F	16	CD	45	L3	5-ASA
CD-18	F	17	CD	30	L3	5-ASA, AZA, CS
CD-19	M	13	CD	32.5	L3	5-ASA, AZA, CS
CD-20	M	10	CD	17.5	L3	5-ASA, AZA, CS
CD-21	F	15	CD	5	L1	5-ASA, AZA
CD-22	F	17	CD	57.5	L3	5-ASA, CS
CD-23	M	4	CD	15	L1	5-ASA
CD-24	F	16	CD	25	L3, L4	5-ASA, AZA, CS
CD-25	M	12	CD	80	L3, L4	5-ASA, AZA, CS, IFX
CD-26	F	12	CD	62.5	L1	5-ASA, AZA, CS
CD-27	M	12	CD	45	L3	5-ASA, AZA, CS
CD-28	F	10	CD	32.5	L3	5-ASA, AZA, CS
CD-29	M	14	CD	52.5	L2	5-ASA, AZA, IFX
CD-30	M	15	CD	45	L3, L4, p	5-ASA, AZA
CD-31	F	16	CD	37.5	L1	5-ASA, AZA
CD-32	F	16	CD	40	L3, L4	5-ASA, AZA
CD-33	M	9	CD	27.5	L3	5-ASA, AZA, CS
CD-34	M	15	CD	60	L3, L4	5-ASA, AZA, CS, IFX
CD-35	M	7	CD	37.5	L1	5-ASA, AZA, CS
CD-36	M	9	CD	25	L3	5-ASA
CD-37	M	10	CD	15	L3	5-ASA, AZA, CS
CD-38	M	17	CD	30	L1	5-ASA, AZA, CS
CD-39	F	12	CD	22.5	L2, p	5-ASA, AZA, CS
CD-40	F	6	CD	25	L1	5-ASA
CD-41	F	17	CD	50	L3, L4, p	5-ASA, AZA, CS
UC-1	F	16	UC	60	LC	5-ASA

Table 1. Cont.

Patient ID	Gender	Age	Diagnosis	PCDAI/ PUCAI	Location of inflammation	Medications
UC-2	F	12	UC	10	PC	5-ASA
UC-3	M	17	UC	10	PC	5-ASA
UC-4	F	9	UC	40	PC	5-ASA, CS
UC-5	M	12	UC	35	PC	5-ASA, CS
UC-6	F	18	UC	30	PC	5-ASA, CS
UC-7	F	16	UC	45	LC	5-ASA
UC-8	F	17	UC	40	PC	5-ASA
UC-9	M	13	UC	40	LC	5-ASA, CS
UC-10	F	12	UC	30	LC	5-ASA, CS
UC-11	F	16	UC	20	LC	5-ASA, CS
UC-12	F	12	UC	10	LC	5-ASA, CS
UC-13	M	7	UC	25	LC	5-ASA
UC-14	F	15.5	UC	40	PC	5-ASA
UC-15	F	16	UC	40	PC	5-ASA, CS, AZA, IFX
UC-16	F	18	UC	30	LC	5-ASA
UC-17	M	17	UC	30	PC	5-ASA, CS
UC-18	F	13	UC	65	LC	5-ASA, CS
UC-19	F	17	UC	40	LC	5-ASA

CD — Crohn's disease, UC — ulcerative colitis, L1 — distal 1/3 ileum ± limited cecal disease, L2 — colonic, L3 — ileocolonic, L4 — upper disease, p — perianal disease, PC — pancolitis, LC — left side colitis, 5-ASA — 5-aminosalicylic acid, AZA — azathioprine, CS — corticosteroids, IFX — infliximab.

There were no statistically significant differences in sex and age between the study groups (median age of patients with CD and UC was 14 years, and median age of controls was 14.5 years; P value = 0.75 in Kruskall-Wallis ANOVA test). The study population included 41 (44.56%) children with CD, 19 (20.65%) children with UC, and 32 (34.78%) controls. Half of UC patients presented with pancolitis and clinical activity of illness assessed according to PUCAI (Pediatric Ulcerative Colitis Activity Index) ranged from 10 to 65 points (mean 33.5, SD ± 14.61). Patients with CD had various types of the disease, including perianal lesions (n = 8), inflammatory lesions of varied locations, and lesions causing clinically irrelevant strictures (n = 3). The clinical activity of illness assessed according to PCDAI (Pediatric Crohn Disease Activity

Index) ranged from 5 to 57.5 points (mean 30.7, SD ± 17.38). None of the patients required surgical intervention during study period.

Assessment of biochemical markers of fibrosis in respective periods revealed significant differences between the study groups (Table 2). 19 patients at week 3rd and 11 at weeks 6th to 8th were lost to follow up because they did not volunteer for follow-up visits at particular periods of time.

Table 2. Concentration of YKL40 and PIIINP (ng/mL).

	Study groups									
	Crohn's disease			Ulcerative colitis			Control			Correlations
	N	Median	QRange	N	Median	QRange	N	Median	QRange	p
YKL at 0	41	34.56	40.21	19	39.50	30.60	32	36.30	27.89	0.96
YKL at 14-21 day	28	37.05	36.28	13	30.50	36.77	0	—	—	0.85
YKL at 6-8 week	19	40.30	45.07	11	24.70	43.81	0	—	—	0.68
PIIINP at 0	41	1013.73	1997.88	19	78.30	723.80	32	399.38	940.29	0.06
PIIINP at 14-21 day	28	748.72	1855.46	13	124.50	411.50	0	—	—	0.37
PIIINP at 6-8 week	**19**	**1076.48**	**1509.80**	**11**	**53.10**	**675.45**	**0**	—	—	**0.01**

YKL-40/CHI3L1 — a 40-kilodalton glycoprotein also referred to as chitinase 3 like-1 — CHI3L1, PIIINP — N-terminal propeptide of type III procollagen

PIIINP levels were significantly higher in children with CD than in children with UC (median levels at baseline were 1013.73 ng/mL vs 78.30 ng/mL, respectively; P value = 0.06 in Kruskall-Wallis ANOVA test; median levels after 6–8 weeks of treatment were 1076.48 ng/mL vs 53.10 ng/mL, respectively; P value = 0.01).

When levels of YKL-40/CHI3L1 were compared in patients with CD and patients with UC, no significant differences were found. YKL-40/CHI3L1 levels slightly increased in CD patients (from 34.56 ng/ml to 40.30 ng/ml) and decreased in UC patients (from 39.50 ng/ml to 24.70 ng/ml) (see Fig. 1A and Fig. 1B).

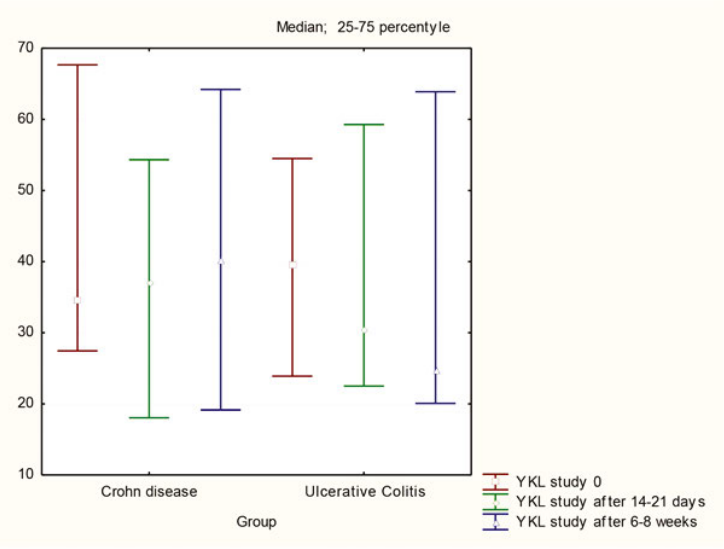

Fig. 1A. Distribution of YKL40 (ng/mL) in patient with CD and UC. Comparison of groups. YKL-40/ CHI3L1 — a 40-kilodalton glycoprotein also referred to as chitinase 3 like-1 — CHI3L1.

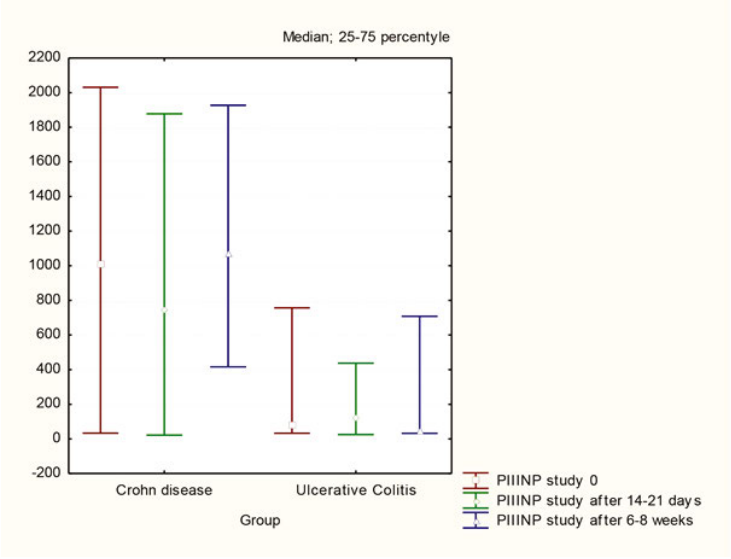

Fig. 1B. Distribution of PIIINP (ng/mL) in patient with CD and UC. Comparison of groups. PIIINP — N-terminal propeptide of type III procollagen.

Discussion

IBD is a group of diseases of still unclear etiology associated with activation of intestinal immune system as a result of abnormal interactions between the host and its microbiome. CD and UC differ with locations of lesions and depth of inflammatory infiltrate. In patients with UC the inflammatory infiltrate causes remodeling of superficial layers of mucosa, while in patients with CD it leads to full-thickness remodeling of the intestinal wall. Fibrosis is a nonspecific response to inflammation. In patients with UC it is limited to intestinal mucosa, while in patients with CD it affects the whole thickness of intestinal wall and leads to intestinal strictures. Fibrosis is closely related to mucosal repair [5–7]. Inflammatory response cells (monocytes, neutrophils) remove the damaged tissues and at the same time produce active immune response factors, such as interleukins (IL), mainly IL-1, IL-6 and tumor necrosis factor (TNF) alpha, that activate fibroblasts and thus initiate mucosal repair processes (reepithelization) such as angiogenesis, fibrogenesis and lymphogenesis. Reepithelization is associated with accumulation of extracellular matrix (ECM) composed mainly of mesenchymal cells (Mcs), including fibroblasts and myofibroblasts. There are several hypotheses on the role of fibrosis in IBD [8–12]. Some of them assume that excessive deposition of ECM is related to tissue remodeling and may lead to tissue fibrosis; moreover it is hypothesized that collagen metabolism is increased both in CD as in inflamed tissue in UC [13, 14]. Data related to the changes in PIIINP level, the propeptide that takes part in intestinal fibrosis and in the turnover of the key ECM components – type I and type III collagen – are inconsistent in patients with CD [15–17]. In Wu *et al*. study it has been established that PIIINP (key component of ECM) can help in determining risk of uncomplicated inflammatory disease converting to stricturing in children with CD. In this study, ECM has been identified as a predictive factor of fibrostenotic complications in patients with CD [18].

A study by Sazouk revealed that in the early inflammatory phase fibrocytes differentiate to fibroblasts, which take part in early fibrosis and increase inflammation by production of TNF alpha [19]. Therefore, it is important to establish whether anti-inflammatory treatment may stop these adverse outcomes related to tissue remodeling and prevent fibrosis. YKL-40/CHI3L1 is a glycoprotein produced by activated macrophages and neutrophils during inflammation; it is also produced by chondrocytes and synovial cells. YKL-40/CHI3L1 is a growth factor stimulating fibroblasts and endothelial cells (fibrogenesis, angiogenesis). YKL-40/CHI3L1 is one of chitinases binding chitin and heparin, and therefore it is currently more commonly known under the name of CHI3L1. Chitinases are enzymes generally typical for organisms that produce chitin, such as insects, fish, shellfish, and other marine organisms, and not for mammals.

Although the last ones do not produce chitin, they may periodically or constantly produce chitinases, which role in mammals is still unclear. It is thought that YKL-40/CHI3L1 is produced by healthy humans. However, it is present in the case of numerous inflammatory conditions, including rheumatoid arthritis or hepatitis, as well as in neoplasms, such as colorectal cancer. In patients with IBD YKL-40/CHI3L1 is present in macrophages of epithelium and lamina propria [20]. YKL is released by activated macrophages and neutrophils and promotes myofibroblast-induced collagen secretion [21]. YKL-40/CHI3L1 and other chitinases produced by mammals play a key role in the pathogenesis of IBD, asthma and other inflammatory diseases. Chitinases are a very important factor in the innate immune response against intestinal microorganisms. Their abnormal overproduction may paradoxically stimulate chronic inflammation.

Our study revealed that during 6–8-week course of treatment with 5-ASA and/or corticosteroids and/or azathioprine patients with CD and UC presented with different levels of YKL-40/CHI3L1 and PIIINP. Fibrosis was more pronounced in patients with CD and demonstrated by an increasing trend for both YKL-40/CHI3L1 and PIIINP during 6–8-week follow-up regardless of the treatment administered during this time. The patterns of YKL-40/CHI3L1 and PIIINP levels in patients with UC were different, with lower absolute values and levels decreasing in the course of treatment.

It is difficult to compare our data with those of other authors, because of very limited number of studies investigating this issue in children [4]. Aomatsu *et al.* find out fecal CHI3L1 as a useful marker that helps to differentiate between IBD and healthy controls with specificity and sensitivity over 80%. Similarly as in our study levels of CHI3L1 were higher in CD compared to UC patients. Basing on study results authors concluded that fecal CHI3L1 can be used as a predictor of severity and activity of the illness [22]. In adult patients results of serum concentration of YKL-40/CHI3L1 are conflicting as fare as its correlation with disease activity is concerned [2, 3, 23]. Alike in our study Vind *et al.* demonstrate that in patients with UC, YKL-40/CHI3L1 diminish during treatment, while in Crohn's patients values are higher and remind elevated also in inactive individuals. In our patients with CD levels of PIIINP were elevated independently on the pharmacological treatment. De Simone *et al.* observed significant reduction in PIIINP levels after 6 month after surgical intervention [24].

Substantial data indicate that treatment used in patients with IBD does not prevent fibrosis. Cytokines and growth factors (TGF-beta, IGF-1, GF-1) released by macrophages, lymphocytes, mast cells or mesenchymal cells contribute to fibrosis by the synthesis of ECM, proliferation of mesenchymal (fibrogenous) cells and therefore, according to the opinion of Lund, no effective anti-fibrotic treatment of IBD is currently available [15, 25].

Ineffectiveness of treatment may be associated with crossing the point of no return, where fibrosis cannot be stopped by anti-inflammatory, immunomodulating

or biological treatment [26]. Some reports indicated that immunosuppressive treatment in patients with risk factors of fibrosis does not lead to significant reduction in the development of strictures [27]. In clinical practice, levels of PIIINP and YKL-40/CHI3L1 are not routinely measured. Moreover, no recommendations on their use in the diagnostics or unequivocal statements on their usefulness are available, and their sensitivity and specificity is not known [28].

From a practical point of view it is important to establish whether a stricture found in a patient with CD is inflammatory (this is associated with a greater chance of resolution during effective treatment) or fibrotic (in such case no effective drugs are available) [29]. Even the effectiveness of the promising biological anti-TNF alpha therapy is probably limited to the former group of patients [30]. PIIINP is a routinely used prognostic factor of liver fibrosis, and it is also useful in chronic cardiovascular diseases associated with fibrosis (hypertension), chronic fibrotic skin diseases or pulmonary diseases, and other conditions [31–33]. The use of PIIINP in adult patients with IBD is controversial because of a chronic course of the disease and high risk of comorbidities that may also be associated with fibrosis. On the contrary, in the case of IBD in young patients with no comorbidities the usefulness of this marker is higher. We attempted to answer the question, whether in children with IBD, YKL-40/CHI3L1 and PIIINP may be used as serological biomarkers allowing for prediction of strictures and monitoring of the outcomes of treatment.

Undoubtedly, among the limitations of our study, we should mention the uneven size of the studied groups, variations in location and clinical activity within both groups of patients, and the loss of a significant number of patients in follow-up.

Our study including in the final analysis 60 children with IBD allows for drawing preliminary conclusions and suggesting future research. Because of a limited sample size our results are of general character and may be used to indicate further directions for research that would include a baseline type of the disease, its severity, type of treatment and its results.

Conclusions

Fibrosis is more pronounced in patients with CD compared to those with UC. PIIINP reflects these differences significantly better than YKL-40/CHI3L1. Further research is necessary to assess long-term perspective of fibrosis in patients with CD, as well as possible differences depending on the type and severity of the disease, and the treatment used.

In patients with UC fibrosis seems to have low intensity and reveal a tendency to resolution over several weeks of treatment. It must still be proven that including YKL-40/CHI3L1 and PIIINP in the diagnostics of IBD may be useful in equivocal cases that are an important problem in pediatric patient population.

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S. Pieczarkowski and K. Kowalska-Duplaga equally contributed to the conception and design of the research, analysis and interpretation of the data; P. Tomasik and P. Kwinta contributed to the conception of the research and statistical analysis; A. Wędrychowicz, S. Pieczarkowski, K. Kowalska-Duplaga, K. Fyderek and A. Stochel-Gaudyn contributed to the acquisition of the data; S. Pieczarkowski and K. Kowalska-Duplaga drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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